

1/16

Figure 1A  
Binding of the mab A76-A/C7 (type GD-1) to a MUC1  
30-mer depending on the glycosylation to the PDTR motif

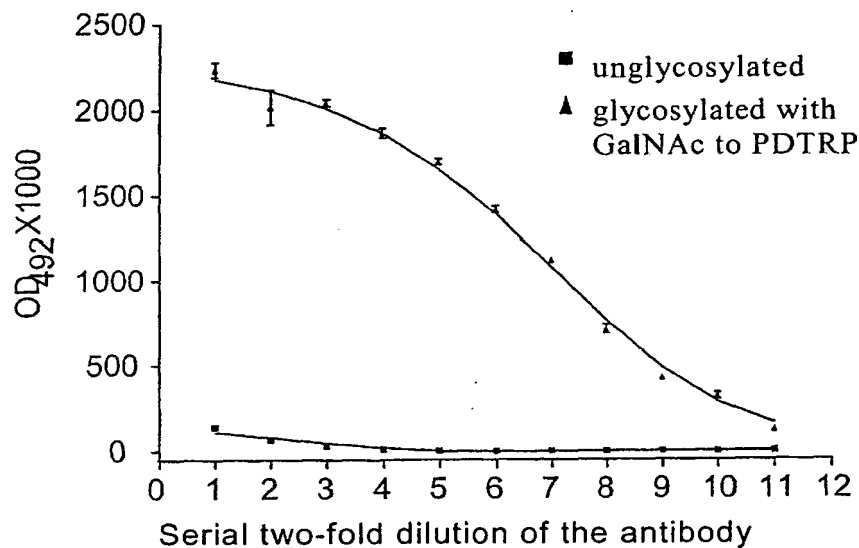
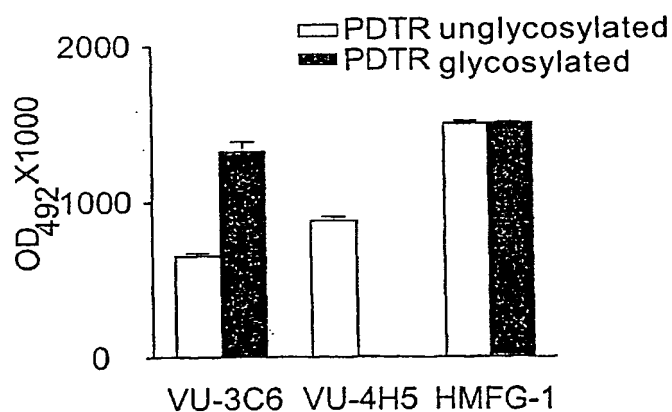


Figure 1B  
Binding pattern of mab against MUC1 of the epitope with GalNAc:  
VU-3C6 (type GD-2), VU-4H5 (type iGD), HMFG-1 (type GI)

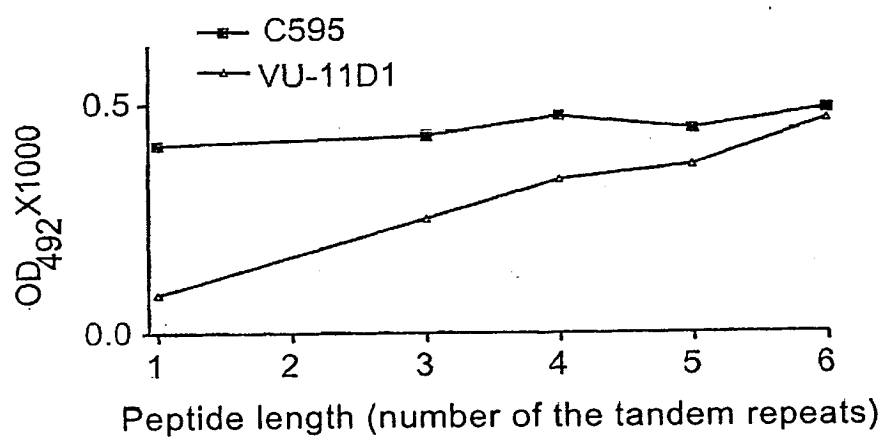


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2/16

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Figure 2



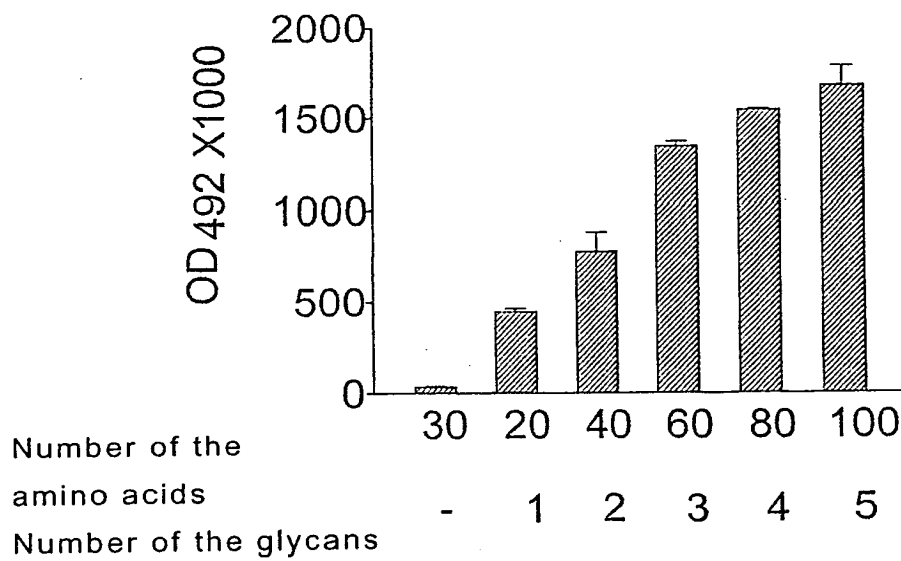
12/13/87

3/16

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**Figure 3A**

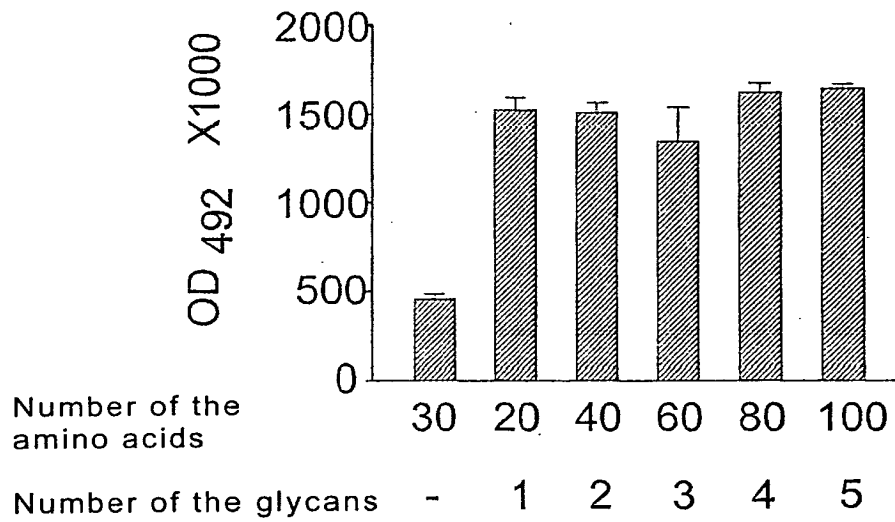
Binding of the mab A76-A/C7 to glycosylated MUC1 peptides of different length (1-5 tandem repeats) (glycosylated with Tn to PDTR motif)



4/16

**Figure 3B**

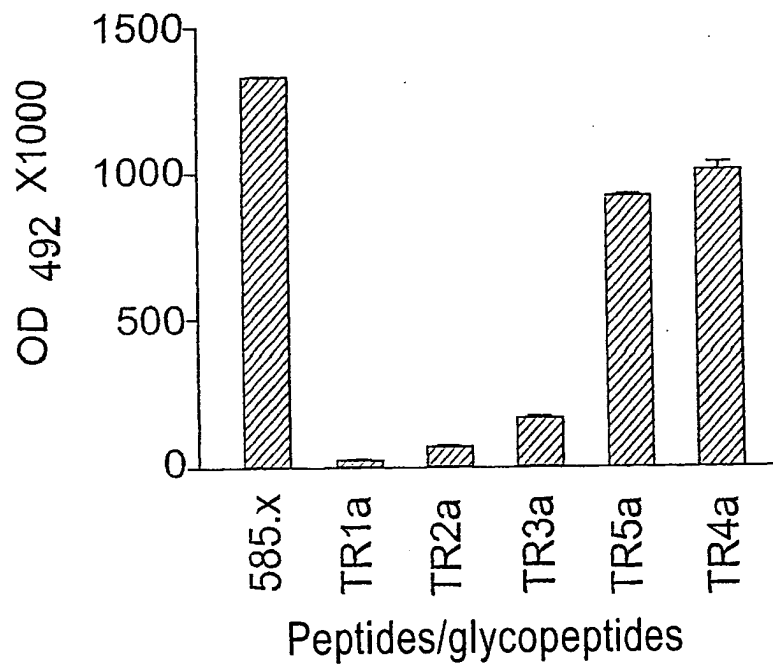
Binding of the mab Mc5 to glycosylated MUC1 peptides of different length  
(1-5 tandem repeats)  
(glycosylated with Tn to PDTR motif)



5/16

**Figure 4**

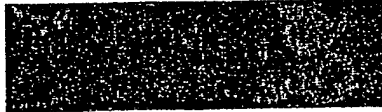
Binding of the mab VU-4H5 to glycosylated MUC1 peptides of different length (1-5 tandem repeats) (glycosylated with Tn to PDTR motif)



6/16

**Figure 5A**

- 1) ZR-75-1 cells before accumulation with A76-A/C7 and cloning

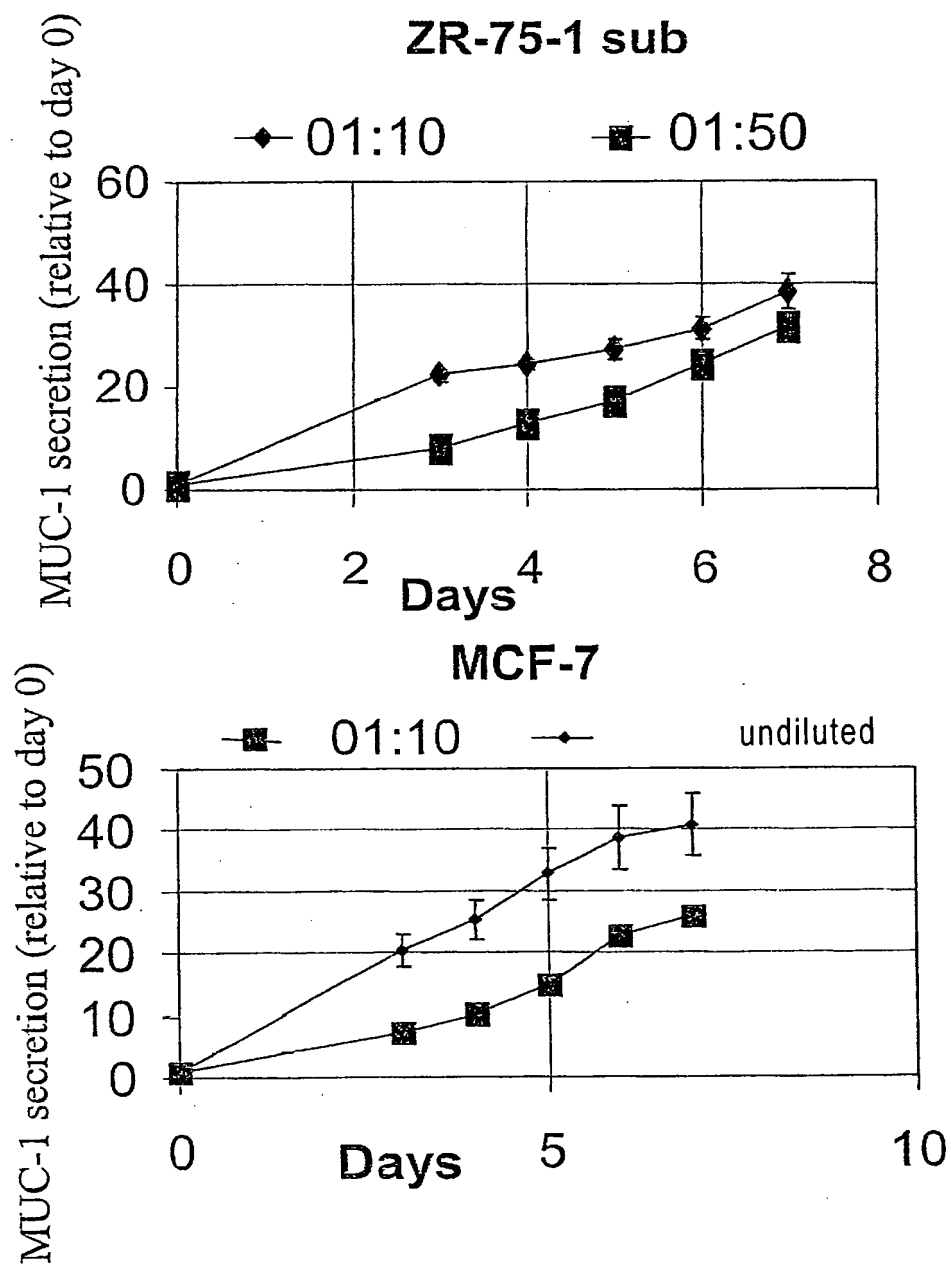


- 2) ZR-75-1 cells after accumulation with A76-A/C7 and cloning (ZR-75-1-sub)

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7/16

Figure 5 B

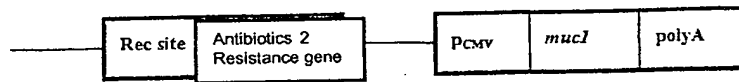


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8/16

Figure 5C (1)

## A. Expression vector



B. Sequenz of the human muc1 cDNA, which were cloned into the expression vector. The primers which were used for the amplification of the cDNA from ZR-75-1 cDNA are marked in green.

```

1  GAATTCCCTG GCTGCTTGAA TCTGTTCTGC CCCCTCCCCA CCCATTTCTCCTG
   CTTAAGGGAC CGACGAACCT AGACAAGACG GGGGAGGGGT GGGTAAAGTG
      +10      +20      +30      +40
   CACGAGCATGACACGGGGCA CCCAGTCTCC TTTCTTCTG CTGCTGCTCC
51  -----
   GTGGTGGTAC TGTGGCCCGT GGGTCAGAGG AAAGAAGGAC GACGACGAGG
      +10      +20      +30      +40
   TCACAGTGCT TACAGTTGTT ACAGGTTCTG GTCATGCAAG CTCTACCCCA
101  -----
   AGTGTCACGA ATGTCAACAA TGTCCAAGAC CAGTACGTTG GAGATGGGGT
      +10      +20      +30      +40
   GGTGGAGAAA AGGAGACTTC GGCTACCCAG AGAAGTTCAG TGCCCGAGCTC
151  -----
   CCACCTCTTT TCCTCTGAAG CCGATGGGTC TCTTCAAGTC ACGGGTCGAG
      +10      +20      +30      +40
   TACTGAGAAG AATGCTGTGA GTATGACCAG CAGCGTACTC TCCAGCCACA
201  -----
   ATGACTCTTC TTACGACACT CATACTGGTC GTCGCATGAG AGGTCGGTGT
      +10      +20      +30      +40
   GCCCCGGTTC AGGCTCCTCC ACCACTCAGG GACAGGATGT CACTCTGGCC
251  -----
   CGGGGCCAAG TCCGAGGAGG TGGTGAGTCC CTGTCCTACA GTGAGACCGG
      +10      +20      +30      +40
   CCGGCCACGG AACCAGCTTC AGGTTTCAGCT GCCACCTGGG GACAGGATGT
301  -----
   GGCCGGTGCC TTGGTCAAG TCCAAGTCGA CGGTGGACCC CTGTCCTACA
      +10      +20      +30      +40
   CACCTCGGTC CCAATCACCA GGCCAGCCCT GGGCTCCACC ACCCCGCCAG
351  -----
   GTGGAGCCAG GGTCAAGTGG CCGGTCGGGA CCCGAGGTGG TGGGGCGGTC
      +10      +20      +30      +40
   CCCACGATGT CACCTCAGCC CCGGACAACA AGCCAGCCCC GGGCTCCACC
401  -----
   GGGTGCTACA GTGGAGTCGG GGCCTGTTGT TCGGTGCGGG CCCGAGGTGG
      +10      +20      +30      +40
   GCCCCCCCAG CCCACGGTGT CACCTCGGCC CCGGACACCA GGCCGCCCCC
451  -----
   CGGGGGGGTC GGGTGCCACA GTGGAGCCGG GGCCTGTGGT CCGGCGGGGG
      +10      +20      +30      +40
   GGGCTCCACC GCCCCCCCAG CCCACGGTGT CACCTCGGCC CCGGACACCA
501  -----
   CCCGAGGTGG CGGGGGGGTC GGGTGCCACA GTGGAGCCGG GGCCTGTGGT
      +10      +20      +30      +40
   GGCCGCCCCC GGGCTCCACC GCGCCCGCAG CCCACGGTGT CACCTCGGCC
551  -----
   CCGGCGGGGG CCCGAGGTGG CGCGGGCGTC GGGTGCCACA GTGGAGCCGG
  
```

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9/16  
Figure 5C (2)

```

+10      +20      +30      +40
601 CCGGACACCA GGC CGGCCCGG GGGCTCCACC GGGGGGGG CCGATGGTGT
*****
GGCTGTGGT CCGGCCGGGG CCGGAGGTGG CGGGGGGGTC GGTACCACA
+10      +20      +30      +40
651 CACCTCGGCC CCGGACAACA GGGCCGCCTT GGCCTCCACC GGGCCTCCAG
*****
GTGGAGCCGG GGCCTGTTGT CCGGGCGGAA CCGCAGGTGG CGGGGAGGTC
+10      +20      +30      +40
701 TCCACAATGT CACCTCGGCC TCAGGCTCTG CATCAGGCTC AGCTTCTACT
*****
AGGTGTTACA GTGGAGCCGG AGTCCGAGAC GTAGTCCGAG TCGAAGATGA
+10      +20      +30      +40
751 CTGGTGACCA ACGGCACCTC TGCCAGGGCT ACCACAACCC CAGCCAGCAA
*****
GACCACGTGT TGCCGTGGAG ACGGTCCCGA TGGTGTGGG GTCGGTCGTT
+10      +20      +30      +40
801 GAGCACTCCA TTCTCAATTC CCAGCCACCA CTCTGATACT CCTACCACCC
*****
CTCGTGAGGT AAGAGTTAAG GGTGGTGGT GAGACTATGA GGATGGTGGG
+10      +20      +30      +40
851 TTGCCAGCCA TAGCACCAAG ACTGATGCCA GTAGCACTCA CCATAGCACG
*****
AACGGTCGGT ATCGTGGTTC TGA CTACGGT CATCGTGAGT GGTATCGTGC
+10      +20      +30      +40
901 GTACCTCCTC TCACCTCCTC CAATCACAGC ACTTCTCCCC AGTTGTCTAC
*****
CATGGAGGAG AGTGGAGGAG GTTAGTGTCG TGAAGAGGGG TCAACAGATG
+10      +20      +30      +40
951 TGGGGTCTCT TTCTTTTCC TGTCTTTTCA CATTTCAAAC CTCCAGTTTA
*****
ACCCAGAGA AAGAAAAGG ACAGAAAAGT GTAAAGTTG GAGGTCAAAT
+10      +20      +30      +40
1001 ATTCTCTCT GGAAGATCCC AGCACCGACT ACTACCAAGA GCTGCAGAGA
*****
TAAGGAGAGA CCTTCTAGGG TCGTGGCTGA TGATGGTTCT CGACGTCTCT
+10      +20      +30      +40
1051 GACATTTCTG AAATGTTTTT GCAGATTTAT AAACAAGGGG GTTTTCTGGG
*****
CTGTAAAGAC TTTACAAAAA CGTCTAAATA TTTGTTCCCC CAAAAGACCC
+10      +20      +30      +40
1101 CCTCTCCAAT ATTAAGTTCA GGCCAGGATC TGTGGTGGTA CAATTGACTC
*****
GGAGAGGTTA TAATTCAAGT CCGGTCCTAG ACACCACCAT GTTAAGTCTG
+10      +20      +30      +40
1151 TGGCCTTCCG AGAAGGTACC ATCAATGTCC ACGACGTGGA GACACAGTTC
*****
ACCGGAAGGC TCTTCCATGG TAGTTACAGG TGCTGCACCT CTGTGTCAAG
+10      +20      +30      +40
1201 AATCAGTATA AAACGGAAGC AGCCTCTCGA TATAACCTGA CGATCTCAGA
*****
TTAGTCATAT TTGCGCTTCG TCGGAGAGCT ATATTGGACT GCTAGAGTCT
+10      +20      +30      +40
1251 CGTCAGCGTG AGTGATGTGC CATTTCCTTT CTCTGCCAG TCTGGGGCTG
*****
GCAGTCGCAC TCACTACACG GTAAAGGAAA GAGACGGGTC AGACCCCGAC

```

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10/16  
Figure 5C (3)

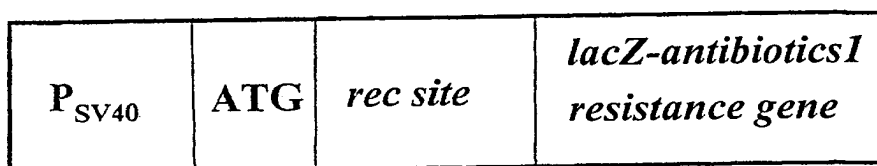
```

          +10      +20      +30      +40
GGGTGCCAGG CTGGGGCATC GCGGTGCTGG TGCTGGTCTG TGTTCGGTT
1301*****
CCCACGGTCC GACCCCGTAG CGCGACGACC ACGACCAGAC ACAAGACCAA
          +10      +20      +30      +40
GCGCTGGCCA TTGTCTATCT CATTGCCTTG GCTGTCTGTC AGTGCCGCGG
1351*****
CGCGACCGGT AACAGATAGA GTAACGGAAC CGACAGACAG TCACGGCGGC
          +10      +20      +30      +40
AAAGAACTAC GGGCAGCTGG ACATCTTTCC AGCCCGGGAT ACCTACCATC
1401*****
TTTCTTGATG CCCGTCGACC TGTAGAAAGG TCGGGCCCTA TGGATGGTAG
          +10      +20      +30      +40
CTATGAGCGA GTACCCCAAC TACCACACCC ATGGGCGCTA TGTGCCCCCT
1451*****
GATACTCGCT CATGGGGTGG ATGGTGTGGG TACCCGCGAT ACACGGGGGA
          +10      +20      +30      +40
AGCAGTACCG ATCGTAGCCC CTATGAGAAG GTTCTGTCAG GTAATGGTGG
1501*****
TCGTCATGGC TAGCATCGGG GATACTCTTC CAAAGACGTC CATTACCACC
          +10      +20      +30      +40
CAGCAGCCTC TCTTACACAA ACCCAGCAGT GGCAGCCACT TCTGCCAACT
1551*****
GTCGTCGGAG AGAATGTGTT TGGGTCGTCA CCGTCGGTGA AGACGGTTGA
          +10      +20      +30      +40
TGTAGGGGCA CGTCGCCCTC TGAGCTGAGT GGCCAGCCAG TGCCATTCCA
1601*****
ACATCCCCGT GAGCGGAGACTGCAATCA GCGGTCGGTC ACGGTAAGGT
          +10      +20      +30      +40
CTCCACTCAG GGCTCTCTGG GCCAGTCCTC CTGGGAGCCC CCACCACAAC
1651-----
GAGGTGAGTC CCGAGAGACC CGGTCAGGAG GACCCTCGGG GGTGGTGTG
          +10      +20      +30      +40
ACTTCCCAGG CATGGAATTC C
1701-----
TGAAGGGTCC GTACCTTAAG G

```

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11/16

**Figure 5D (1)****A. Rec site vector****Features of the stably transfected host cells:**

- Integration of the recombination site in connection with the lacZ antibiotic<sup>1</sup> resistance fusion gene
  - Resistance against the antibiotic<sup>1</sup>, expression of the lacZ gene ( $\beta$ -galactosidase activity)
  - The transfectants differ with respect to the strength of the expression of the fusion gene (chromosomal positioning effect) depending on the integration site of the recombination site and of the fusion gene in the chromosome. Detection by  $\beta$ -galactosidase activities differing in strength.
-

12/16

**Figure D (2).**

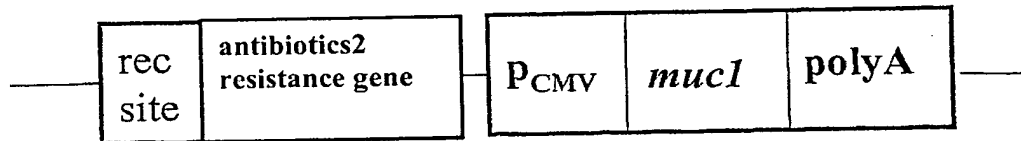
- A. Integration of the muc1 cDNA via the rec site into the ZR-75-1 genome

Recombinase  
Expression vector



+

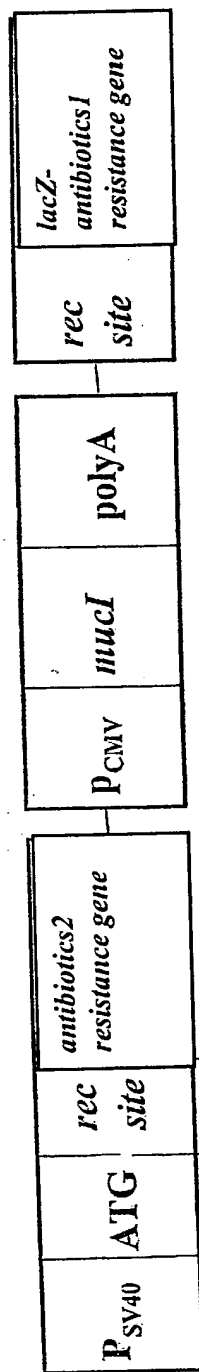
Muc1 expression vector with recombination site (rec site)



Product of the  
side-specific  
recombination

13/16

Figure D (3)

**Features of the stably transfected host cells:**

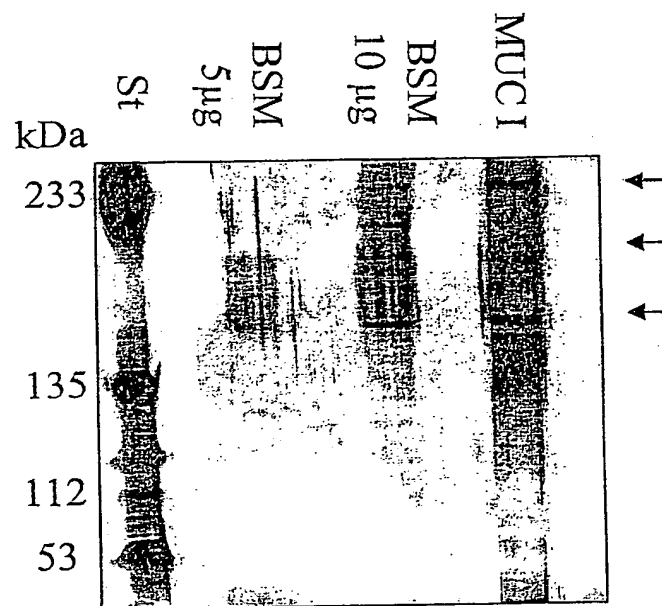
- Resistance against antibiotic 2
- Sensitivity to antibiotic 1,  $\beta$ -galactosidase inactive
- High expression of the recombinant *muc1* gene due to the strong promoter and the chromosomal positioning effect

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14/16

**Figure 5E**

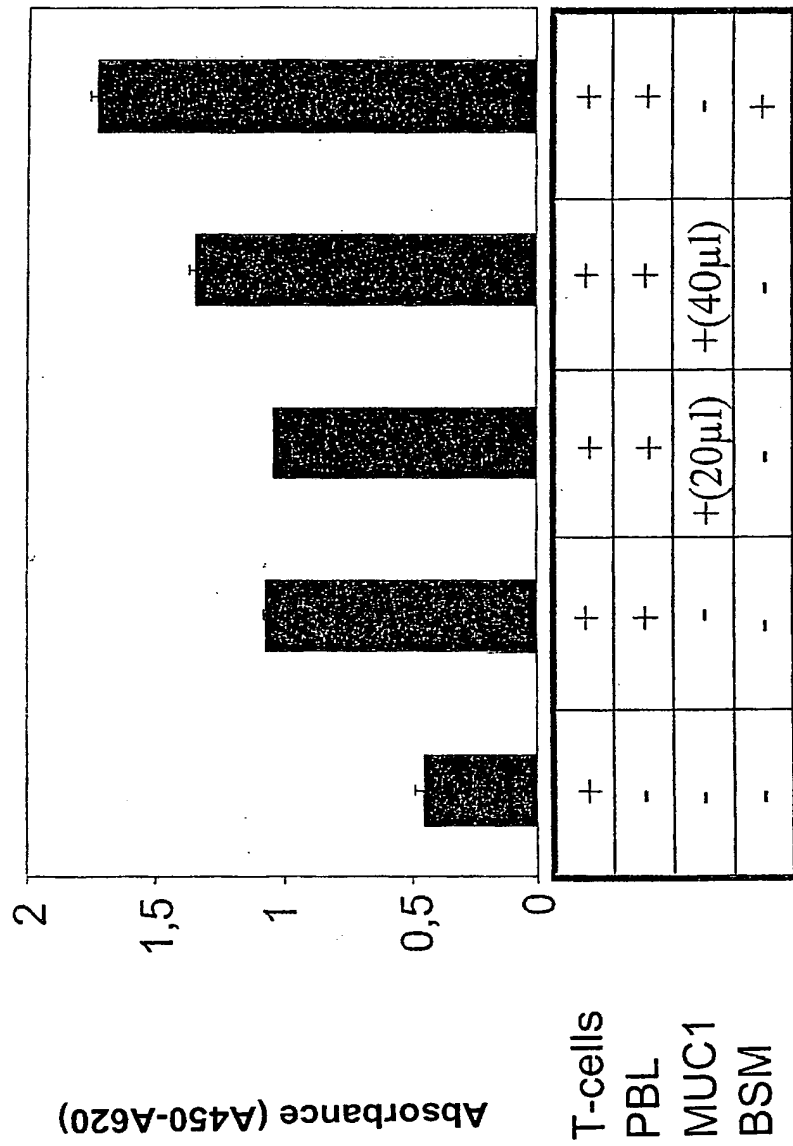
BSM - bovine submaxillary mucin 1  
MUC1 - Mucin 1 from cell supernatants



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15/16

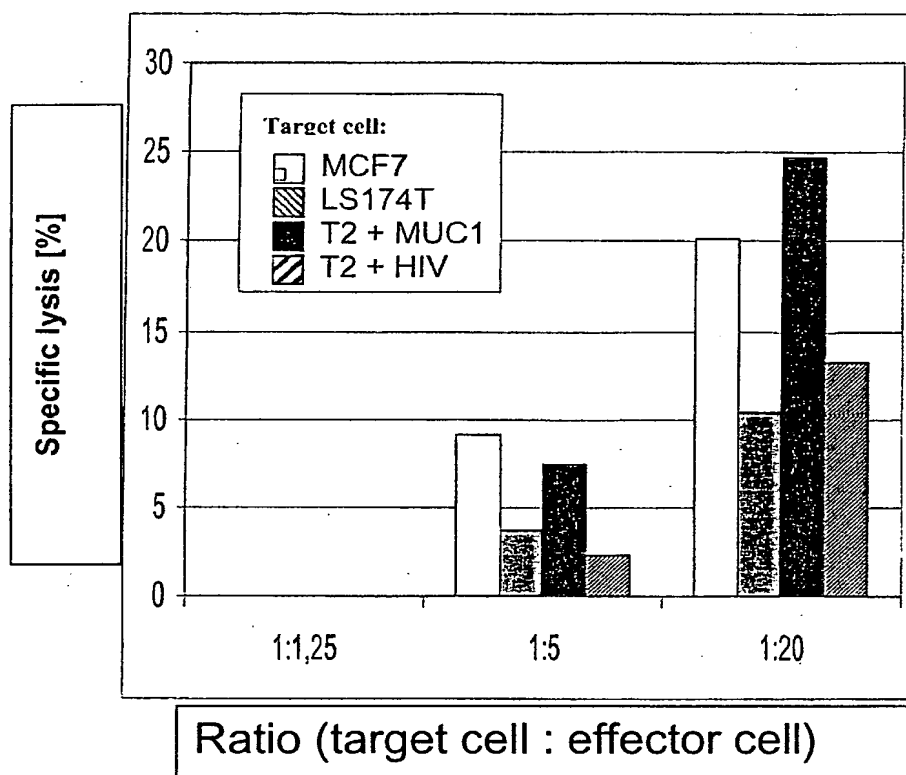
Figure 5F



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16/16

Figure 6



	% SR
MCF7	8,86
LS147T	7,58
T2 + MUC1	3,18
T2 +HIV	4,37

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